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(11) CA 2 385 593 (13) A1

(40) 05.04.2001
(43) 05.04.2001

(12)

(21) 2 385 593

(22) 19.08.2000

(51) Int. Cl.[7]: A61K 49/00, C07K 16/18

(85) 22.03.2002

(86) PCT/EP00/08121

(87) WO01/023005

(30) 199 47 559.8 DE 24.09.1999

(71) SCHERING AKTIENGESELLSCHAFT,
M□llerstrasse 178
D-13342, BERLIN, XX (DE).

(72) DINKELBORG, LUDGER (DE).
LICHA, KAI (DE).
SCHIRNER, MICHAEL (DE).

(74) MARKS & CLERK

(54) CONJUGUES ANTICORPS-COLORANT CONTRE DES STRUCTURES CIBLES DE L'ANGIOGENESE POUR UNE REPRESENTATION PEROPERATOIRE DE BORD DE TUMEUR

(54) ANTIBODY DYE CONJUGATES FOR BINDING TO TARGET STRUCTURES OF ANGIOGENESIS IN ORDER TO INTRAOPERATIVELY DEPICT TUMOR PERIPHERIES

(57)

The invention relates to antibody dye conjugates which are suited for binding to structures of newly formed vessels and to the their use for interoperatively depicting pathological angiogenesis.

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CA 2385593 A1 2001/04/05

(21) 2 385 593

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2000/08/19
(87) Date publication PCT/PCT Publication Date: 2001/04/05
(85) Entrée phase nationale/National Entry: 2002/03/22
(86) N° demande PCT/PCT Application No.: EP 2000/008121
(87) N° publication PCT/PCT Publication No.: 2001/023005
(30) Priorité/Priority: 1999/09/24 (199 47 559.8) DE

(51) Cl.Int.^{7}/Int.Cl.^{7} A61K 49/00, C07K 16/18

(71) Demandeur/Applicant:
SCHERING AKTIENGESELLSCHAFT, DE

(72) Inventeurs/Inventors:
SCHIRNER, MICHAEL, DE;
LICHA, KAI, DE;
DINKELBORG, LUDGER, DE

(74) Agent: MARKS & CLERK

(54) Titre : CONJUGUES ANTICORPS-COLORANT CONTRE DES STRUCTURES CIBLES DE L'ANGIOGENESE POUR UNE REPRESENTATION PEROPERATOIRE DE BORD DE TUMEUR

(54) Title: ANTIBODY DYE CONJUGATES FOR BINDING TO TARGET STRUCTURES OF ANGIOGENESIS IN ORDER TO INTRAOPERATIVELY DEPICT TUMOR PERIPHERIES

(57) Abrégé/Abstract:
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Abstract

Antibody-dye conjugates that are suitable to bind newly formed vessels to structures and their use for intraoperative visualization of pathological angiogenesis are described.

Antibody-Dye Conjugates for Angiogenesis Target Structures for Intraoperative Tumor Edge Visualization

This invention relates to antibody-dye conjugates that are suitable to bind to structures of newly formed vessels and their use for intraoperative visualization of pathological angiogenesis.

In the adult organism, no new vessel formation takes place, with few exceptions (e.g., the cycle of women of child-bearing age). The new vessel formation can be observed, however, in many diseases. The process of the new vessel formation that takes place here is referred to as angiogenesis and takes place as a response to certain signals.

Angiogenesis is a process that preferably takes place in the edge area of a focus of disease. From the center of the focus of disease, factors are released that diffuse to the edge area of the focus of disease. These factors are also referred to as angiogenesis stimulators. If these angiogenesis stimulators reach the healthy tissue in the edge area of a focus of disease, the vessels previously not integrated in the focus of disease are stimulated to form new vessel buds. The vessels that extend from these vessel buds form a new capillary vascular network in the edge area of the focus of disease. By this process, a suitable nutrient supply for the focus of disease can always be ensured. It has proven especially important that the growth of tumors and their metastases depends on the ability to induce angiogenesis.

Surgical therapy is now a standard measure for treating localized foci of disease. It has obtained great importance in the case of tumor treatment. It has turned out, however, that despite improved surgical techniques, the number of local recurrences is considerable, since the anatomical conditions in the human organism only rarely allow a large-scale removal of the focus of disease. In many organs (e.g., in the brain), large-scale removal must be eliminated to obtain healthy tissue. The risk of damaging healthy organs increases with the degree of radicalness of surgical intervention.

Histological studies of the edge area of the tumor after the completion of surgical tumor removal have shown, however, that a considerable number of tumors cannot be removed completely, and tumor radicals remain in the body. Additional tumor growth and also tumor metastasizing can spring from these tumor radicals. A process that exactly indicates the limits of a disease process with respect to healthy tissue during surgical treatment would allow the focus of disease to be removed completely and to leave the healthy tissue unaffected to a large extent.

Dyes for the visualization of foci of disease are already known (Poon, W. S. et al., J. Neurosurgery (1992) 76: 679-686, Haglund, M. M. et al., Neurosurgery (1996) 38: 308-317). They are preferably removed directly from tumor cells or accumulate unspecifically in the extracellular space of tumors. Since the mechanism can be detected in the concentration even in healthy tissue, the specificity and sensitivity of the substances that are used is low.

Compounds that can be used for intraoperative delineation of the foci of disease by selective visualization of the edge area of a focus of disease are not known to date.

The angiogenesis preferably takes place in the edge area of foci of disease. By visualization of the angiogenesis, the limit in healthy tissue can be visualized. Antibodies for detecting angiogenesis in the focus of disease are already known and are used for visualizing newly formed vessels in the histological tissue section, for detecting various proteins in the focus of disease or as carrier molecules for therapeutic substances.

Antibodies in combination with dyes, so-called antibody-dye conjugates, that can be used for intraoperative delineation of the focus of disease by selective visualization of the edge area of a focus of disease are not known, however.

The object of this invention is therefore to prepare antibody-dye conjugates for the intraoperative tumor edge visualization. The antibodies of the antibody-dye conjugates according to the invention are directed against structures that are specifically for the process of angiogenesis. The antibody-dye conjugates according to the invention comprise dyes that make possible an optical visualization by their concentration.

Since the angiogenesis is made most strongly in the edge area of a focus of disease, it results here in the maximum optical signal.

The antibody-dye conjugates according to the invention are thus suitable to visualize the limits of a focus of disease, the so-called edge area, for healthy tissue by intraoperative,

optical diagnosis. It is consequently made possible to remove the focus of disease completely in leaving healthy tissue largely unaffected.

Antibodies are known that are directed against molecules that are strongly expressed in angiogenetically active tissue and are expressed only to a very small level in the adjoining tissue (WO 96/01653).

Antibodies that are directed against the receptors for vascular growth factors, receptors in endothelial cells to which inflammation mediators bind, receptors in endothelial cells to which matrix molecules bind, and matrix proteins that are expressed specifically in the new vessel formation (Brekken et al., Cancer Res. (1998) 58: 1952-9 and Schold, S. C. Jr. et al., Invest. Radiol. (1993) 28: 488-96) are of special interest in antibody-dye conjugates.

Preferred are antibodies or antibody fragments that are directed against the matrix protein EDB-fibronectin. EDB-fibronectin (EDBFN), also known as oncofetal fibronectin, is a splice variant of the fibronectin, which specifically forms newly formed vessels in the process of angiogenesis. The special advantage of antibodies against the EDB-fibronectin consists in that it does not result in any new formation of EDB-fibronectin in healthy tissue by intraoperative injury in the removal of the focus of disease. In this connection, the specificity is preserved during the surgical intervention. Antibodies against growth factor receptors or inflammation mediators in the endothelial cells, which are also expressed specifically in the

tumor edge area, can be newly formed, however, during the surgical intervention even in healthy tissue near the focus of disease.

Especially preferred in the inventive antibody-dye conjugate are antibody L19 and E8 against the EDB-fibronectin (Viti, F., et al., Cancer Res. (1999) 59: 347-352).

Such antibody-dye conjugates are also the subject matter of this invention.

The known antibodies are conjugated with dyes, whose concentration in the tissue can be optically detected and makes possible the intraoperative delineation of the edge area of a focus of disease.

The advantage of the antibody-dye conjugates according to the invention now consists in the fact that the latter can be used for a selective fluorescence staining of tissues in a neoangiogenetic stage. The fluorescence staining is tumor-specific and yields a fluorescence signal that can be detected in a high signal-to-background ratio.

Antibody-dye conjugates for fluorescence imaging are also known for purposes of percutaneous, non-invasive tumor visualization (Neri, D. et al., Nature Biotechnology (1997) 15: 1271-1275).

Not known, however, are antibody-dye conjugates, which preferably accumulate in the edge area of a focus of disease.

Protein-dye conjugates for intraoperative tumor visualization are also known.

The disadvantage to these conjugates is that especially hypoxic and metabolically undernourished tumor cells take up the conjugates. Since the tissue in the edge area of tumors is well vascularized, however, and in this connection the cells are supplied adequately with oxygen and nutrients, for this very reason adequate accumulation of the known protein-dye conjugates is not possible.

The antibody-dye conjugates according to the invention are largely independent of the metabolic state of the focus of disease, however.

Although the optical detection of the limits of a focus of disease can be carried out in different ways, in general the detection of the dye-specific fluorescence radiation induced by corresponding stimulation light is preferred. Depending on the emission wavelength, in this case the fluorescence can be visually detected directly macroscopically or microscopically and optionally simultaneously recorded digitally by imaging detection systems and visualized on a display.

Fluorescence radiation of the spectral range of 400 to 650 nm is visually detectable. Especially preferred is a wavelength of 450 to 600 nm. The special advantage of the use of the visible range of light consists in the fact that the detection of fluorescence by low technical expense is possible. Stimulation light, which is produced by suitable lasers or laser diodes, is coupled to a fiber optic light guide and is brought in by the latter to the area to be diagnosed. The implementation of the intraoperative tumor edge detection is carried out by large-scale

radiation of the area. The reflected stimulation light is blocked by a filter (e.g., a pair of filtering glasses that are worn by the person making the study), and only the dye-specific fluorescence is observed (macroscopic observation). As an alternative, the detection of the fluorescence can be carried out by an operating microscope (microscopic observation). By the small penetration depth of VIS light in the tissue (a few millimeters), new vascular formations located on the surface can be detected in this way.

Another advantage of the spectral range of visible light exists in the small penetration depth in the tissue and emission from the tissue. The detectable signal consequently is not distorted by signals from deeper portions of tissue and can be assigned specifically to the tissue structures that are visible on the surface.

The subjects of this invention are thus also antibody-dye conjugates, whose dyes induce an optical signal in the visible spectral range of the light.

The use of antibody-dye conjugates with dyes that absorb in the spectral range of near-infrared light (NIR; 600-900 nm) makes possible, however, the detection of new vascular formation in deeper tissue layers (up to 1 cm), since NIR light is absorbed more weakly from tissue and therefore has a larger tissue penetration depth. The observation of fluorescence is visually impossible and can be carried out by CCD-cameras (charge-coupled device camera), which are placed over the tissue area of interest. Both macroscopic and microscopic detection are

possible. The advantage of the use of dyes in the antibody-dye conjugates, which absorb and fluoresce in the NIR-spectral range, then is at work if an evaluation of masked areas (e.g., by blood) is necessary.

From the photophysical standpoint, those dyes that have an absorption maximum within the spectral range of 400 to 800 nm and at least one fluorescence maximum within 500 to 900 nm are suitable for antibody-dye conjugates.

The subjects of this invention are also antibody-dye conjugates that are characterized in that the dye induces a fluorescence signal only with use of a defined wavelength range of the visible or near-infrared light.

Antibody-dye conjugates that comprise dyes with visually detectable fluorescence are, for example, those from the following classes:

fluorescein, fluorescein-isothiocyanate, carboxyfluorescein or calcein,

tetrabromofluoresceins or eosins, tetraiodofluoresceins or erythrosins,

difluorofluorescein, such as, e.g., Oregon Green™ 488, Oregon Green™ 500 or Oregon Green™ 514, carboxyrhodol (Rhodol Green™)-dyes (US 5,227,487; US 5,442,045), carboxyrhodamine dyes (e.g., Rhodamine Green™ dyes) (US 5,366,860),

4,4-difluoro-4-bora-3a,4a-diaza-indacene, such as, e.g., Bodipy FL, Bodipy 493/503 or Bodipy 530/550 and derivatives thereof (US 4,774,339, US 5,187,288, US 5,248,782, US 5,433,896 and US 5,451,663),

cyanine dyes, especially carbocyanines and merocyanines,

coumarin dyes, such as, e.g., 7-amino-4-methylcoumarin,

metal complexes of DTPA or tetraazamacrocyclene (cyclene, pyclene) with terbium or europium or tetrapyrrole dyes, especially porphyrins.

Antibody-dye conjugates that comprise near-infrared dyes are, for example, those from the following classes:

polymethine dyes, such as dicarbocyanine, tricarbocyanine, merocyanine and oxonol dyes (WO 96/17628),

rhodamine dyes,

phenoxazine or phenothiazine dyes,

tetrapyrrole dyes, especially benzoporphyrins, chorines and phthalocyanines.

Preferred near-infrared dyes in the antibody-dye conjugates are the cyanine dyes with absorption maxima of between 700 and 800 nm, especially indodi- and indotricarbocyanines.

Generally preferred are dyes in the antibody-dye conjugates from the above-mentioned classes, which have one or more carboxyl groups, which are coupled to amino groups of antibodies or antibody fragments after chemical activation. Those derivatives that contain maleimido or bromoalkyl radicals are also preferred, so that a covalent coupling to the sulfhydryl group of the amino acid cysteine is carried out.

In addition, dyes that have isothiocyanate groups, which also react with amino groups, are preferred.

Moreover, the dyes in the antibody-dye conjugates must have a high photostability and do not bleach out under irradiation

with light (photobleaching) to ensure a constant signal within the study period.

The subjects of this invention are thus antibody-dye conjugates that preferably accumulate in the edge area of the cell tissue of a focus of disease and thus make the edge area of the focus of disease optically detectable.

In particular subjects of this invention are antibody-dye conjugates of general formula I

$$B-(F)_n \qquad (I),$$

in which

B stands for an antibody or an antibody fragment with high binding to ED-BFN,

F stands for a dye from the class of coumarins, fluoresceins, carboxyfluoresceins, difluorofluoresceins, tetrabromofluoresceins, tetraiodofluoresceins, rhodamines, carboxyrhodamines, carboxyrhodols, 4,4-difluoro-4-bora-3a,4a-diaza-indacenes, polymethine dyes or tetrapyrrole dyes, or the terbium or europium complexes with DTPA or cyclene and its derivatives, and

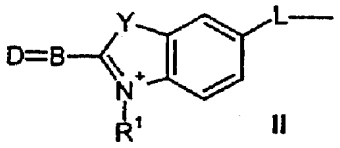
n stands for 1 to 5.

Especially preferred and thus also the subjects of this invention are antibody-dye conjugates, whose dye is a cyanine dye, a merocyanine dye, an oxonol dye, a styryl dye or a squarilium dye.

Especially preferred and thus also subjects of this invention are antibody-dye conjugates in which the dye portion is

a cyanine dye, especially a carbocyanine, dicarbocyanine or tricarbocyanine.

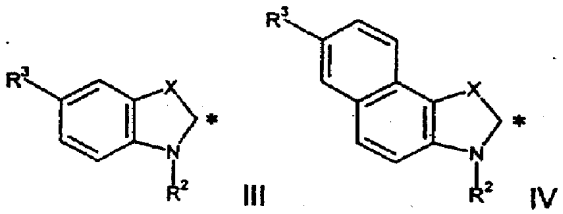
The invention thus relates in particular to those antibody-dye conjugates in which dye $-(F)_n$ of general formula I is a cyanine dye of general formula II



II

in which

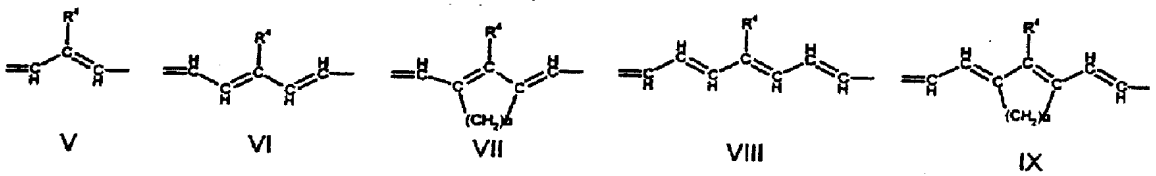
D stands for a radical III or IV



III IV

whereby the position labeled with a star means the interface site with radical B, and

B can stand for group V, VI, VII, VIII or IX



V VI VII VIII IX

in which

R^1 and R^2 mean C_1-C_4 sulfoalkyl, a saturated or unsaturated, branched or linear C_1-C_{50} alkyl chain, which optionally can be substituted with up to 15 oxygen atoms, and/or with up to 3 carbonyl groups, and/or with up to 5 hydroxy groups,

R^3 stands for group $-COOE^1$, $-CONE^1E^2$, $-NHCOE^1$, $-NHCONHE^1$, $-NE^1E^2$, $-OE^1$, $-OSO_3E^1$, $-SO_3E^1$, $-SO_2NHE^1$ or $-E^1$, whereby

E^1 and E^2, independently of one another, stand for a hydrogen atom, C_1-C_4 sulfoalkyl, saturated or unsaturated, branched or straight-chain C_1-C_{50} alkyl, which optionally is interrupted with up to 15 oxygen atoms, and/or up to 3 carbonyl groups, and/or can be substituted with up to 5 hydroxy groups,

R^4 stands for a hydrogen atom or a fluorine, chlorine, bromine or iodine atom,

b stands for 2 or 3,

X stands for oxygen, sulfur or the group $=C(CH_3)_2$ or -(CH=CH)-, and

L stands for a direct bond or a linker, which is a straight-chain or branched carbon chain with up to 20 carbon atoms, which can be substituted with one or more -OH, -COOH, or SO_3 groups and/or optionally can be interrupted in one or more places by an -O-, -S-, -CO-, -CS-, -CONH-, -NHCO-, -NHCSNH-, $-SO_2-$, PO_4- or an -NH group or an aryl ring.

The antibody-dye conjugates according to the invention can be used either alone or in a formulation as pharmaceutical agents.

To use the antibody-dye conjugates as pharmaceutical agents, the latter are brought into the form of a pharmaceutical preparation, which in addition to the antibody-dye conjugate contains pharmaceutical, organic or inorganic inert media that are suitable for enteral or parenteral administration, such as, for example, water, gelatin, gum arabic, lactose, starch, magnesium stearate, talc, vegetable oils, polyalkylene glycols, etc. The pharmaceutical preparations can be present in solid form, for example as tablets, coated tablets, suppositories, capsules or in liquid form, for example as solutions, suspensions or emulsions. They optionally contain, moreover, adjuvants such as preservatives, stabilizers, wetting agents or emulsifiers, salts for altering the osmotic pressure or buffers.

For parenteral use, injection solutions or suspensions, especially aqueous solution of antibody-dye conjugates, are suitable.

As vehicle systems, surface-active adjuvants, such as salts of bile acids or animal or plant phospholipids, but also mixtures of them as well as liposomes or their components can also be used.

For oral use, especially tablets, coated tablets or capsules with talc and/or hydrocarbon vehicles or binders, such as, for example, lactose, corn or potato starch, are suitable. The

application can also be carried out in liquid form, such as, for example, as juice, to which optionally a sweetener is added.

The dosage of the antibody-dye conjugates can vary depending on the method of administration, age and weight of the patient, type and severity of the disease to be treated and similar factors. The applicable dose of the antibody-dye conjugates for detecting limit areas is 0.5-1000 mg, preferably 50-200 mg, whereby the dose can be given as a single dose to be administered once or divided into two or more daily doses.

The above-described formulations and forms for dispensing are also the subjects of this invention.

The invention thus also relates to pharmaceutical agents that comprise one or more antibody-dye conjugates for intraoperative visualization of the edge areas of a focus of disease, whereby the pharmaceutical agents are used either alone or in a mixture with suitable solvents, buffers and/or vehicles.

The antibody-dye conjugates according to the invention are used in the surgical treatment of angiogenesis-dependent diseases, such as malignant tumors and metastases thereof, benign tumors, precancerous tissue changes, endometriosis, hemangiomas and ectopic pregnancies.

Also a subject of this invention is the use of antibody-dye conjugates and agents for intraoperative visualization of foci of disease, especially for microscopic and macroscopic, intraoperative visualization of edge areas of a focus of disease, as well as the use of antibody-dye conjugates for the production of an agent for surgical treatments of angiogenesis-dependent

diseases, such as malignant tumors and metastases thereof, benign tumors, precancerous tissue changes, endometriosis, hemangiomas and ectopic pregnancies.

Production of Dyes

The production of dyes is carried out according to methods that are known in the literature. Suitable dyes for the production of antibody-dye conjugates are dyes of carboxyl groups or isothiocyanate groups for covalent coupling to amino groups of the antibody. Especially preferred in this case are cyanine dyes (Mujumdar, S. R. et al. (1996) 7: 356-362; Flanagan, J. H. et al. (1997) 8: 751-756 and Licha, K. et al. (1996) Proc SPIE Vol. 2927, 192-198).

The dyes with carboxyl groups are activated first by conversion into a reactive ester (e.g., N-hydroxysuccinimide ester) according to methods that are known in the art. Dyes with isothiocyanate groups can be used directly. The reactive derivatives are then brought to reaction in buffer solution or mixtures of organic solvent (e.g., dimethylformamide (DMF) or dimethyl sulfoxide (DMSO)) and buffer solution with the antibody. In this case, a 3- to 100-fold molar excess of dye is used. The unreacted portion is separated by ultrafiltration and/or chromatography after the reaction is completed.

The following dye is also produced by a similar method:

Production Example 1

Bis-1,1'-(4-sulfobutyl)indocarbocyanine-5-carboxylic acid-N-hydroxysuccinimide ester

The production of bis-1,1'-(4-sulfobutyl)indocarbocyanine-5-carboxylic acid is carried out starting from 1-(4-sulfobutyl)-2,3,3-trimethyl-3H-indolenine and 1-(4-sulfobutyl)-2,3,3-trimethyl-5-carboxy-3H-indolenine (Cytometry 10, 11-19, 1989, Talanta 39, 505-510, 1992) in a way similar to methods known in the literature.

For conversion into the N-hydroxysuccinimide ester, 0.1 mmol of dye (67 mg in 10 ml of DMF) is mixed in each case with 0.5 mmol of N-hydroxysuccinimide and dicyclohexylcarbodiimide (DCC) and stirred for 24 hours at room temperature. After 50 ml of ether is added, the precipitated solid is filtered off, dissolved again twice each in a little DDF and precipitated with ether and finally dried in a vacuum (yield 89%).

Production of the Antibody-Dye Conjugate

Production of a bis-1,1'-(4-sulfobutyl)indocarbocyanine conjugate with an L19-antibody

The antibody L19 (1 mg in 1 ml of sodium acetate buffer 50 mmol, pH 8.2)) is mixed with N-hydroxysuccinimide ester (75 µmol of a solution of 4 mg/ml in DMSO) and stirred for 2 hours at room temperature. Purification is done using gel filtration on PD10-cartridges (Pharmacia) and concentration is done using Centricon-

10 tubes (Amicon) while obtaining a solution of about 1 mg/ml of antibody.

Absorption maximum: 555 nm

Fluorescence maximum: 582 nm.

The following example explains the biological usability of the antibody-dye conjugates according to the invention without limiting the latter to the sample application.

Sample Application 1

In vivo fluorescence imaging on tumor-carrying nude mice and microscopic ex-vivo examination of the tumor tissue

The imaging properties of the compounds according to the invention were examined in vivo after injection in tumor-carrying nude mice. For this purpose, 0.1 µmol/kg to 2 µmol/kg of the substance was administered intravenously, and the concentration in the tumor region is observed in a period of 0 to 48 hours. The fluorescence of the substances is induced by irradiation of the animals with light of corresponding wavelength, which is produced monochromatically with a laser (diode laser, solid-state laser) or is filtered out by filter from the polychromatic emission of an Hg or Xe lamp. In the case of the compound that is described in production example 1, light from an Nd:YAG laser of wavelength 540 nm is used for excitation to stimulate the test animal, and the fluorescence radiation at a wavelength of >580 nm is detected by an intensified CCD camera while obtaining whole-body-fluorescence images. In a parallel manner, the fluorescence is detected visually and photographically. Sections are prepared from the tumor material and studied by microscope (Zeiss Axiovert microscope with Cy3-filter set).

After injection of 1 µmol/kg of the antibody-dye conjugate in F9-teratocarcinoma-carrying nude mice mentioned in the

production example, it was possible after four hours to detect an increased fluorescence signal in comparison to normal tissue based on whole-body-fluorescence images.

After preparation of the skin and the topmost tissue layers of the tumor, the fluorescence can be associated with the edge areas of the tumor. The microscopic evaluation of tumor sections produces an elevated fluorescence, which correlates with blood vessels of the tumor edge area.

Claims

1. Antibody-dye conjugates that preferably accumulate in the edge area of the cell tissue of a focus of disease and thus make the edge area of the focus of disease optically detectable, characterized in that the dye is a compound of general formula I

$$B-(F)_n \qquad (I),$$

in which

B stands for an antibody or an antibody fragment with high binding to EDB-fibronectin,

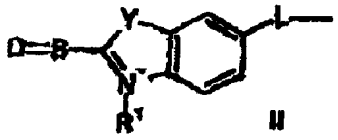
F stands for a dye from the class of coumarins, fluoresceins, carboxyfluoresceins, difluorofluoresceins, tetrabromofluoresceins, tetraiodofluoresceins, rhodamines, carboxyrhodamines, carboxyrhodols, 4,4-difluoro-4-bora-3a,4a-diaza-indacenes, polymethine dyes or tetrapyrrole dyes, or the terbium or europium complexes with DTPA or cyclene and its derivatives, and

n stands for 1 to 5.

2. Antibody-dye conjugates according to claim 1, wherein the dye is a cyanine dye, a merocyanine dye, an oxonol dye, a styryl dye or a squarilium dye.

3. Antibody-dye conjugates according to claim 1, wherein the dye is a cyanine dye such as carbocyanine, dicarbocyanine or tricarbocyanine.

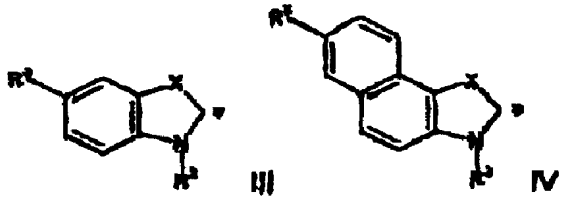
4. Antibody-dye conjugates according to claims 1 to 3, wherein dye $-(F)_n$ of general formula I is a cyanine dye of general formula II



II

in which

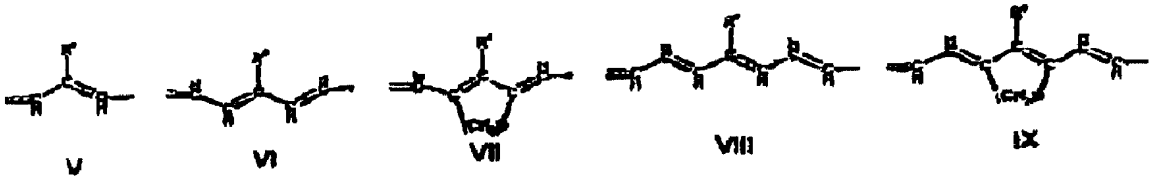
D stands for a radical III or IV



III IV

whereby the position labeled with a star means the interface site with radical B, and

B can stand for group V, VI, VII, VIII or IX



V VI VII VIII IX

in which

R^1 and R^2 mean C_1-C_4 sulfoalkyl, a saturated or unsaturated, branched or linear C_1-C_{50} alkyl chain, which optionally can be substituted with up to 15 oxygen atoms, and/or with up to 3 carbonyl groups, and/or with up to 5 hydroxy groups,

R^3 stands for group -COOE^1, -CONE^1E^2, -NHCOE^1, -NHCONHE^1, -NE^1E^2, -OE^1, -OSO_3E^1, -SO_3E^1, -SO_2NHE^1 or -E^1, whereby

E^1 and E^2, independently of one another, stand for a hydrogen atom, C_1-C_4 sulfoalkyl, saturated or unsaturated, branched or straight-chain C_1-C_{50} alkyl, which optionally is interrupted with up to 15 oxygen atoms, and/or up to 3 carbonyl groups, and/or can be substituted with up to 5 hydroxy groups,

R^4 stands for a hydrogen atom or a fluorine, chlorine, bromine or iodine atom,

b stands for 2 or 3,

X and Y stand for oxygen, sulfur or the group $=C(CH_3)_2$ or -(CH=CH)-,

and

L stands for a direct bond or a linker, which is a straight-chain or branched carbon chain with up to 20 carbon atoms, which can be substituted with one or more -OH, -COOH, or SO_3 groups and/or optionally can be interrupted in one or more places by an -O-, -S-, -CO-, -CS-, -CONH-, -NHCO-, -NHCSNH-, -SO_2-, PO_4- or an -NH group or an aryl ring.

5. Antibody-dye conjugates according to claims 1 to 4, wherein antibodies L19 and E8 are used as antibodies.

6. Antibody-dye conjugates according to claims 1 to 5, wherein the dye in the visible spectral range of the light induces an optical signal.

7. Antibody-dye conjugates according to claims 1 to 5, wherein the dye induces a fluorescence signal only with use of a defined wavelength range of the visible or near-infrared light.

8. Pharmaceutical agent comprising one or more antibody-dye conjugates according to claims 1 to 7 for intraoperative visualization of the edge areas of a focus of disease.

9. Pharmaceutical agent according to claim 8, in a mixture with suitable solvents, buffers and/or vehicles.

10. Use of the antibody-dye conjugates and agents according to claims 1 to 9 for intraoperative visualization of foci of disease.

11. Use of antibody-dye conjugates according to claims 1 to 9 for intraoperative visualization of the edge areas of a focus of disease.

12. Use of antibody-dye conjugates according to claims 1 to 9 for microscopic and macroscopic intraoperative visualization of the edge areas of a focus of disease.

13. Use of antibody-dye conjugates according to claims 1 to 7 for the production of an agent for surgical treatment of angiogenesis-dependent diseases, such as malignant tumors and metastases thereof, benign tumors, precancerous tissue changes, endometriosis, hemangiomas and ectopic pregnancies.